From Page No. 43

F cutter → my primer made what I had ordered but IT frame shifted everything!

Ordered a new PCR primer

Genentech, Inc.

SYNTHETIC DNA REQUEST (A-7702) V

PROJECTOR	EXTENSION	LAB NO.	PROJECT NAME OR NUMBER	DATE NEEDED	AMT. NEEDED
WILL BARON	2650	10231	1713		

LIST SEQUENCE(S) 5' → 3' & NOTE AS SUCH
SIZE & FRAGMENT NAME:

1x 37mer
WB-1
5' GTA·CGG·TGA·CCC·C GG·CGG·TCG·GGC·
TCC·CCT·CGG·GCT·T·3 [redacted]

Tyro P28

PLEASE INDICATE BY "**" FRAGMENT(S) TO BE CLONED

FRAGMENT USE: ☐ PRIMER ☐ PROBE ☐ PCR ☒ GENE CONSTRUCTION ☐ LINKER/ADAPTOR
☐ MUTAGENESIS ☐ OTHER (SPECIFY):

SPECIAL REQUESTS:

SUBMITTED BY: Will Baron DATE: [redacted] APPROVED: Mark Vasser DATE: 3-24-93

Need to set up for Northern analysis on Melanie Mark's cell line + tissue northern

For probe
RD
5 µl pGEM-3Z (w/ 600 bp HPTK6 5')
2 µl 10x RA
1 µl Sac I
12 µl H2O
20

Inc 37°C O/N.

To Page No.

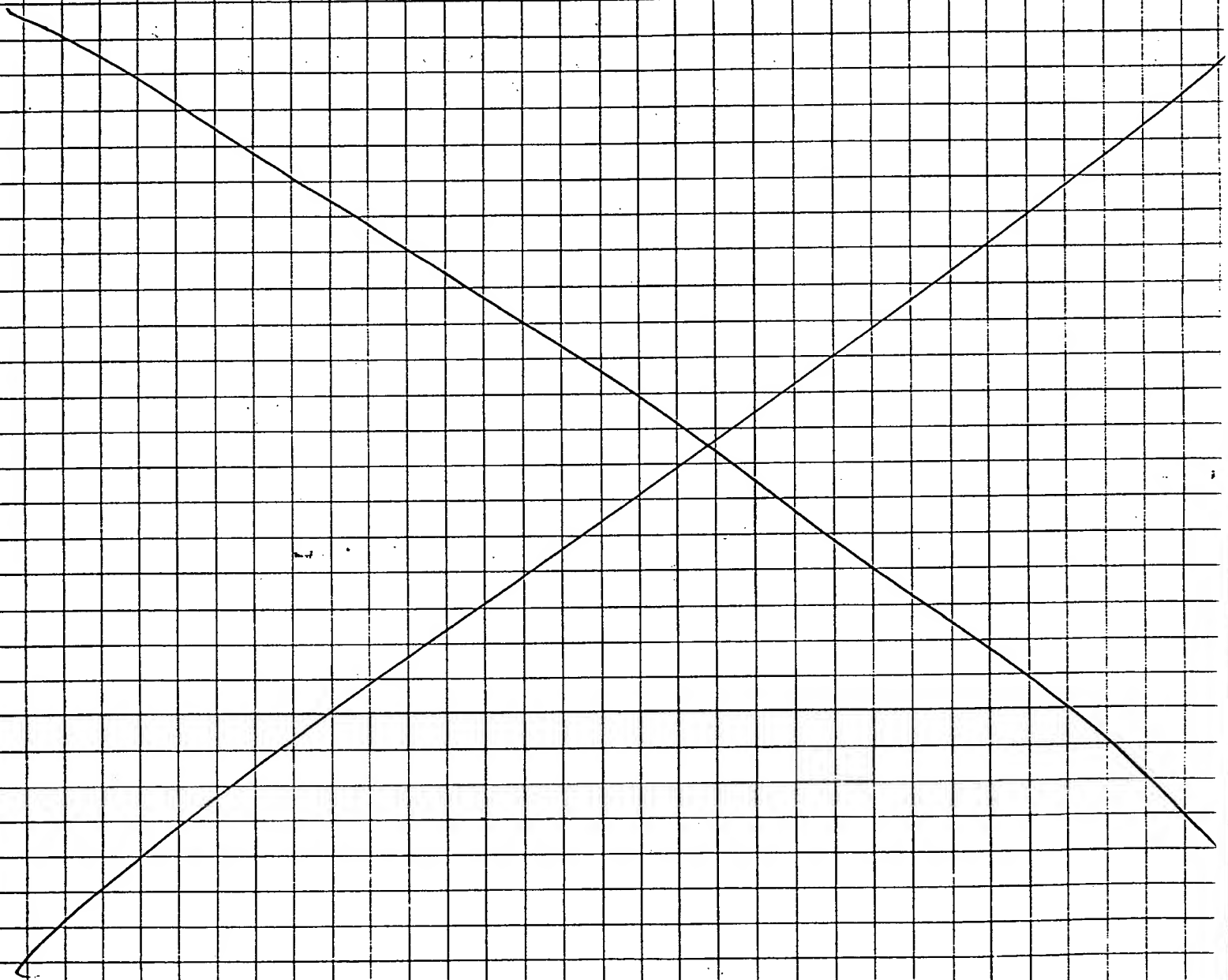
Witnessed & Understood by me, | Date | Invented by Will Baron | Date [redacted]

Recorded by [redacted]

From Page No. 47

Picked up primers to make new fusion construct of HPTK6

Ran PCR as on p. 17 O/N.
used Tyro P13 + Tyro P23(new)



To Page N

Witnessed & Understood by me,	Date	Invented by	Date [redacted]
		Recorded by Will Bacon	

From Page No. 51

Did magic PCR prep on O/N FNS PCR

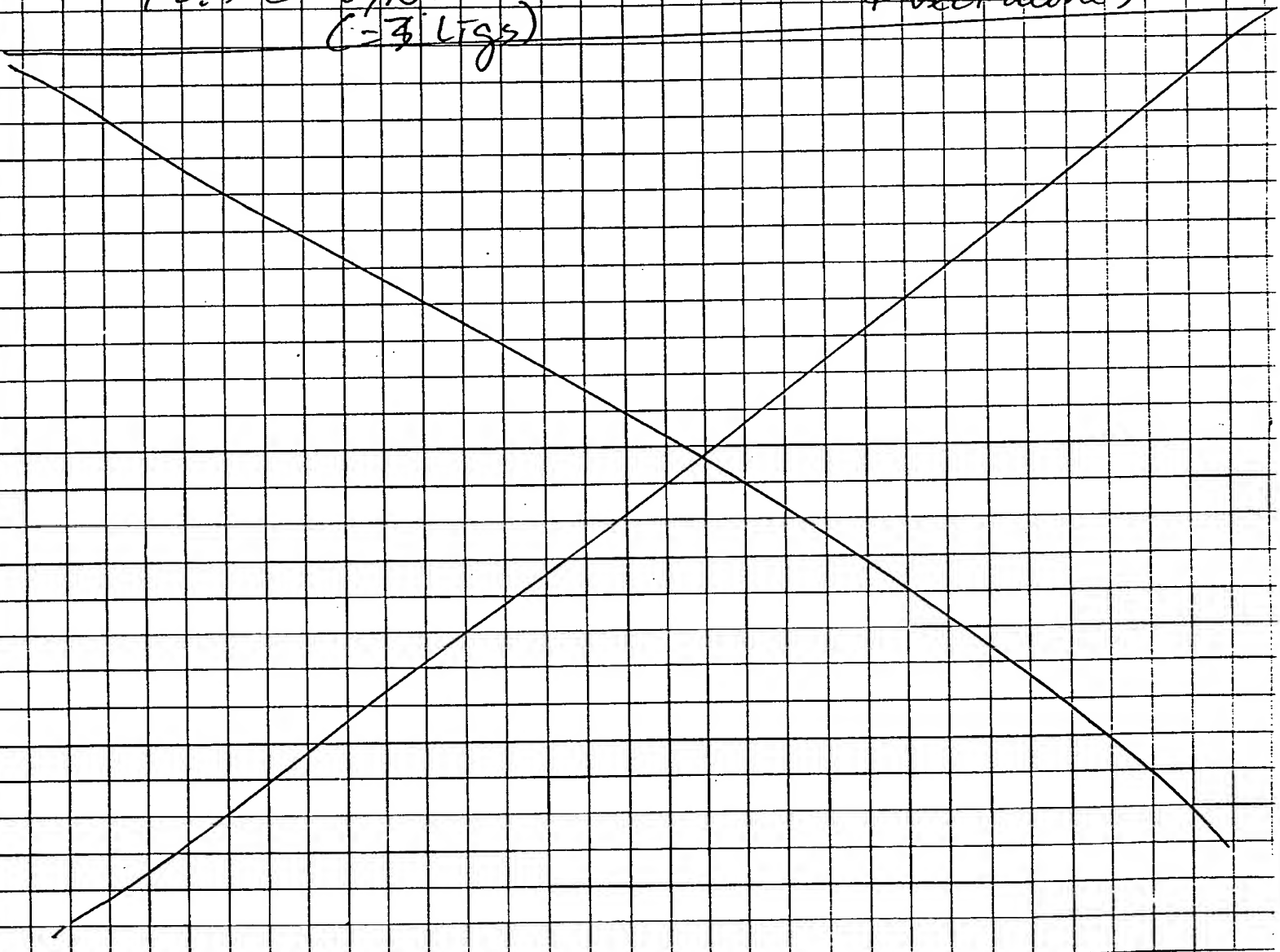
Digested w/ Avr II & BstE II

Clean-up done

Did 3 part ligation w/ 5' insert + SKB /cH2cH (Hi/Lo conc & vect alone)

(2.5°C O/N.

(~3 Ligs)



To Page No

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by WM Bacon	[redacted]

From Page No. 52

Transformed O/N SK@/CH2CH3/HPTK6 Fus Ligations into competent E. coli

Plated each onto 5 x 100mm LB carb 50 plates, (control onto 1 plate)

Inc all (11) 37°C O/N.

To Page

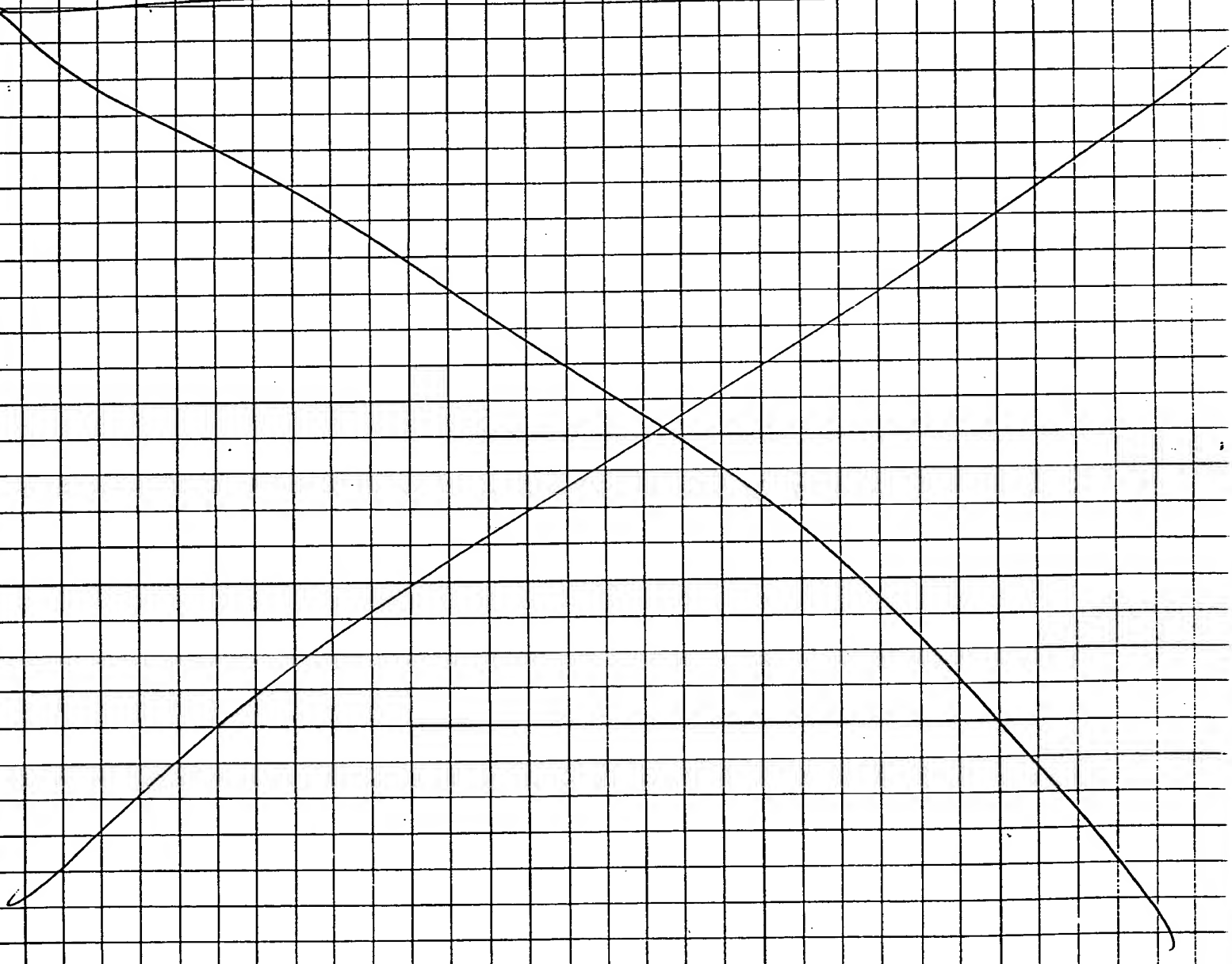
Witnessed & Understood by me, | Date | Invented by WW Bacon | Recorded by | Date [redacted]

Checked O/N FMS transformation plates

Got 19 colonies

Started 19 x 5ml LB carb50 MP's + Master

Inc all 37°C O/N.



To Page No.

From Page No. 54 (→RDGH2CH3/HPTK6 FUS)

Did Magic MP's on O/N cultures (stored masters 4°C)

RD's

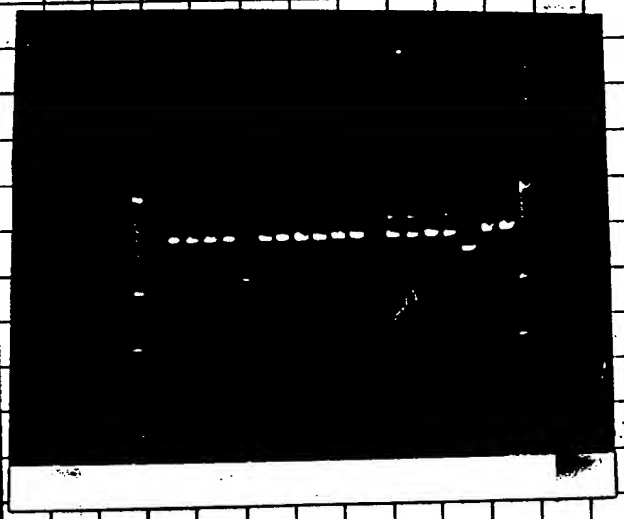
Per rxn
- 2µl MP DNA
- 2µl 10x B
- 0.5µl HindIII
- 15.5µl H_2O
- 20

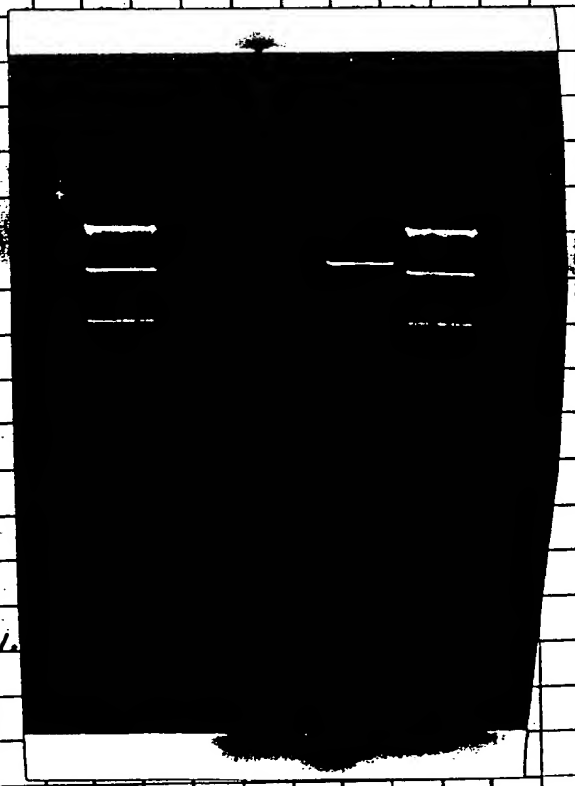
37°C – 1.5 hrs (+ 4µl dye)

Ran 15µl each on 0.7% agarose (1x TBE)

No real ones!

Ran Individual compon
(eg ligation mix)
on 2% agarose (1x TBE,





No vector or 3' PCR insert seen.

Re-ran PCR as on p. 48 O/N

Redid 3 part Ligation O/N.

To Page

Witnessed & Understood by me,	Date	Invented by WM Bauer	Date [redacted]
		Recorded by	

Page No. 55

Transformed new HPTK6/Fus 3part Lig.
Plated onto 11 x 100mm LB carb 50 → Inc 37°C o/N

Extracted O/N PCR
ran 10µl on 2% agarose (1xTBE)
Also ran 10µl of OLD Fus 3' PCR

PCR not working.

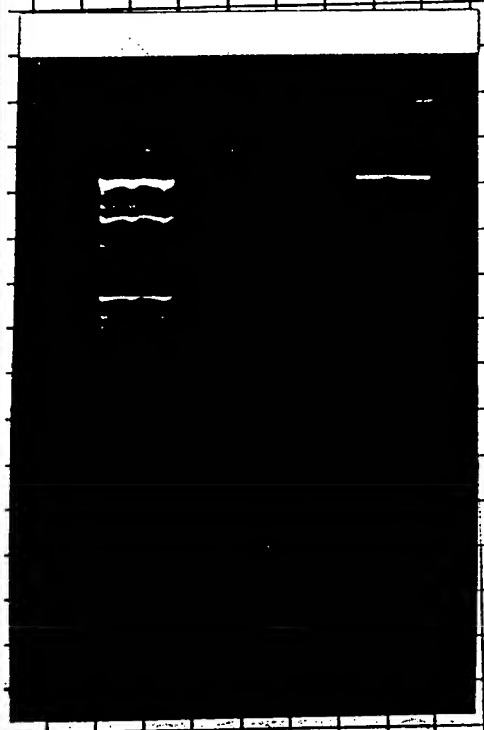
Must not have worked in 1st try either → This is why I didn't get any fusion transformants

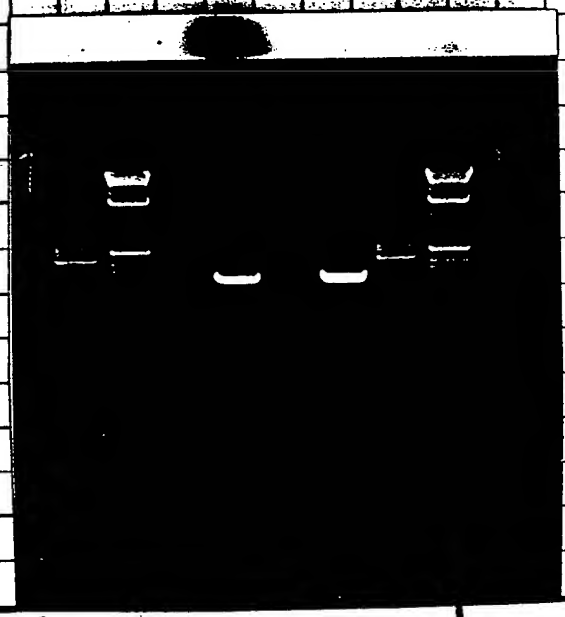
17/48
Re ran PCR as on p. ~~55~~ except I used pRK5/HPTK6 as template

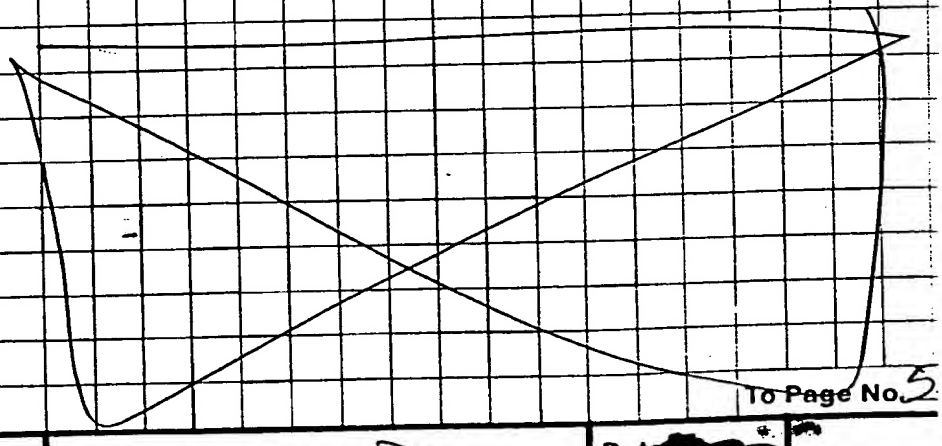
(also ran BspII w/ Bsp15/21 as a ~300bp PCR control)
(same conditions as on p 17)

Extracted & ran 20µl each on 2% 3:1 Nusieve/GTG (1x TBE)

New primer (Tyro P28) appears to be no-good.







To Page No. 5

Witnessed & Understood by me,

Date

Invented by Wm Bowen

Recorded by

Date [redacted]

Genentech, Inc.

SYNTHETIC DNA REQUEST A- 7657 ✓

REQUESTOR	EXTENSION	LAB NO.	PROJECT NAME OR NUMBER	DATE NEEDED	ART NEEDED
Will Baron	2650	10231	1713		

LIST SEQUENCE(S) 5' → 3' & NOTE AS SUCH
SIZE & FRAGMENT NAME:

PLEASE INDICATE BY "*" FRAGMENT(S) TO BE CLONED

Tyro P28, 37mer

⑤ GTA·CGG·TGA·CCC·CGG·GGG·TCG·GGC·
TCC·CCT·CGG·GCT·T[redacted] 3'

(Remake of A29X, WD-1)

Resubmitted P28 for new synthesis

To Page

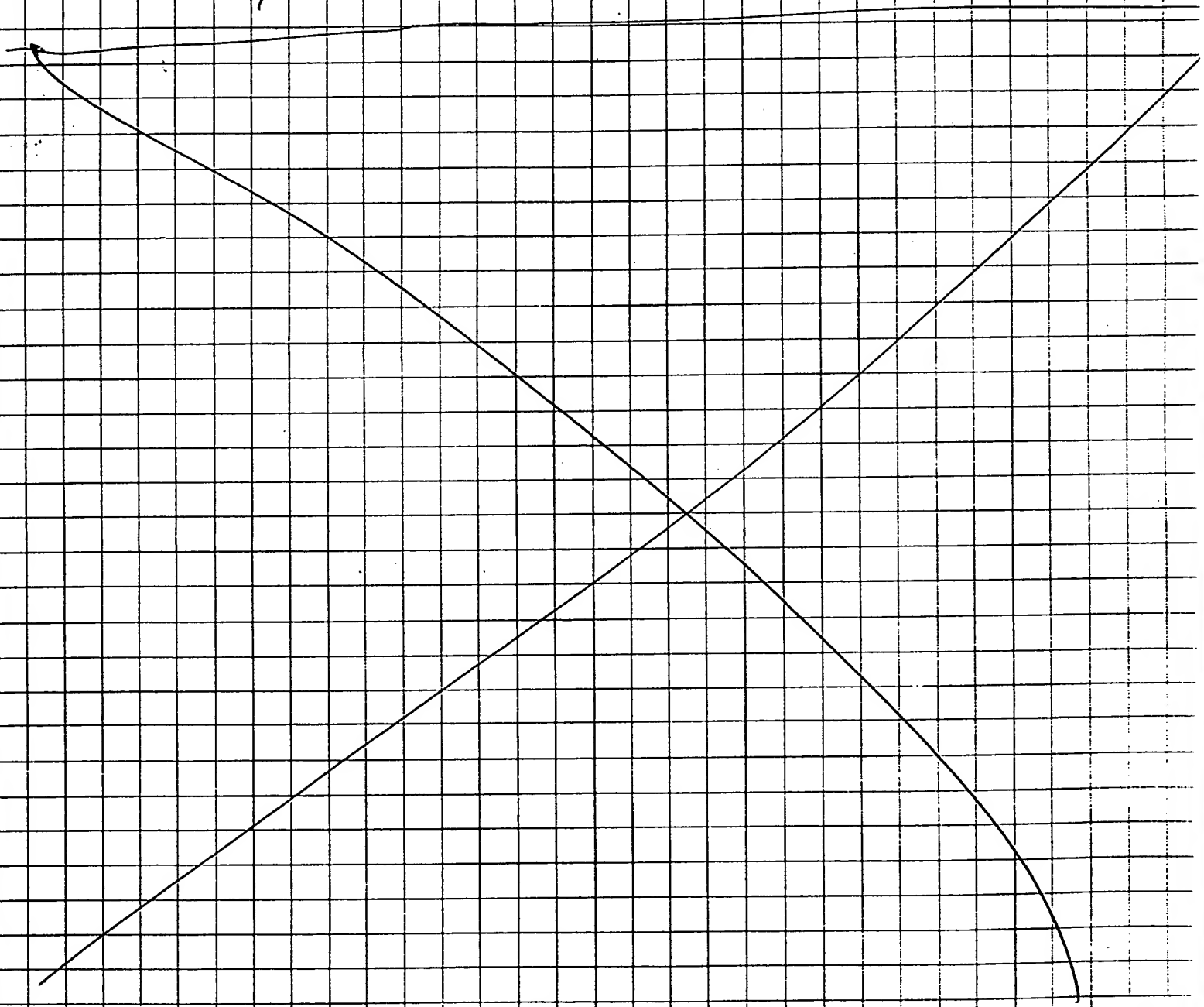
Witnessed & Understood by me,	Date	Invented by W. M. Baron	Date [redacted]
		Recorded by [redacted]	

From Page No. 57

Picked up remade Tyra P28

Re-ran fus PCR's as previously.

Ran O/N.



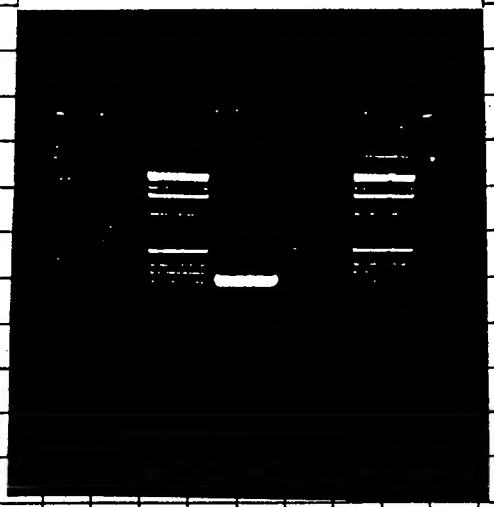
To Page N

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by	[redacted]

From Page No. 58

Extracted O/N PCR's 1 x 100µl CHCl3

Ran 20µl each on 2% agarose. (1 x TBE)



P28 is not working

Try varying the Mg^{2+} conc

Re ran P13/P28 rxns using

(.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 9 & 10 mM Mg^{2+}

~~Ran~~ Also changed conditions to "Touchdo

1st annealing @ 65°C → ended @ 55°C
2 cycles at each temp → 10 @ 55°C

All 30" 72°C extension

Ran O/N.

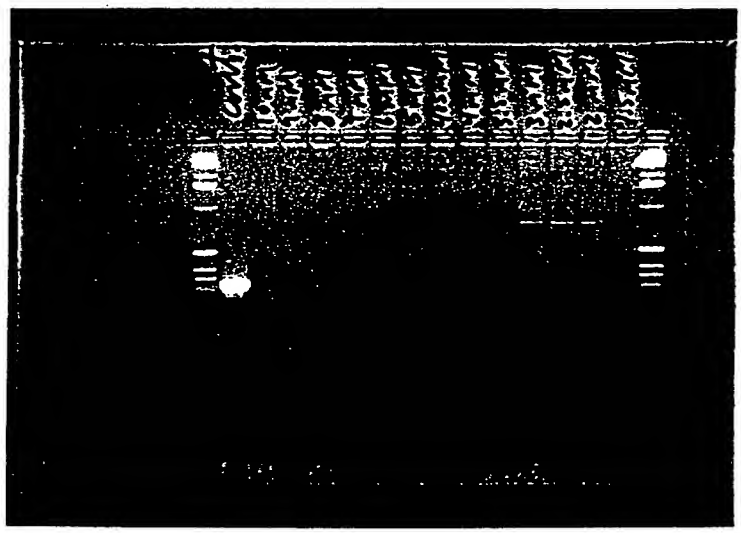
To Pag

Witnessed & Understood by me,	Date	Invented by WM Bacon	Date [redacted]
		Recorded by	

from Page No. 59

Extracted O/N PCR's 1 x 100µl CHCl3 each

Run 20µl each on 2% agarose (1 XTBE)



All failed.

The only conclusion I can reach at this time is that there is something weird about primer 28.

Ordered 3 new primers to work up from P28 position.

Genentech, Inc.
Genentech, Inc.

SYNTHETIC DNA REQUEST (A- 7726) V

REQUESTOR	EXTENSION	LAB NO.	PROJECT NAME OR NUMBER	DATE RECEIVED	ANT. NEEDED
WILL BARON	2650	10231	17-13		

LIST SEQUENCE(S) 5' → 3' & NOTE AS SUCH
SIZE & FRAGMENT NAME:
3 x 35 mers

PLEASE INDICATE BY "*" FRAGMENT(S) TO BE CLONED

① Tyro P29
* GTA·CGG·TGA·CCG·CGG·TCG·GGC·TCC·CCT·CGG·GCT·TG·3' [redacted].

② Tyro P30
* GTA·CGG·TGA·CCG·TCG·GGC·TCC·CCT·CGG·GCT·TGG·CC·3' [redacted].

③ Tyro P31
* GTA·CGG·TGA·CCG·GGC·TCC·CCT·CGG·GCT·TGG·CCA·CG·3' [redacted].

FRAGMENT USE: ☐ PRIMER ☐ PROBE ☐ PCR ☒ GENE CONSTRUCTION ☐ LINKER/ADAPTOR ☐ MUTAGENESIS ☐ OTHER (SPECIFY):

SPECIAL REQUESTS

AUTHORIZED BY	DATE	APPROVED	DATE
WM Baron	4/9/93	Mark Knuth	4-12-93

To Page N

Witnessed & Understood by me,	Date	Invented by WM Baron	Date [redacted]
		Recorded by	

From Page No. 60

Picked up new HPTK6 PCR fusion primers

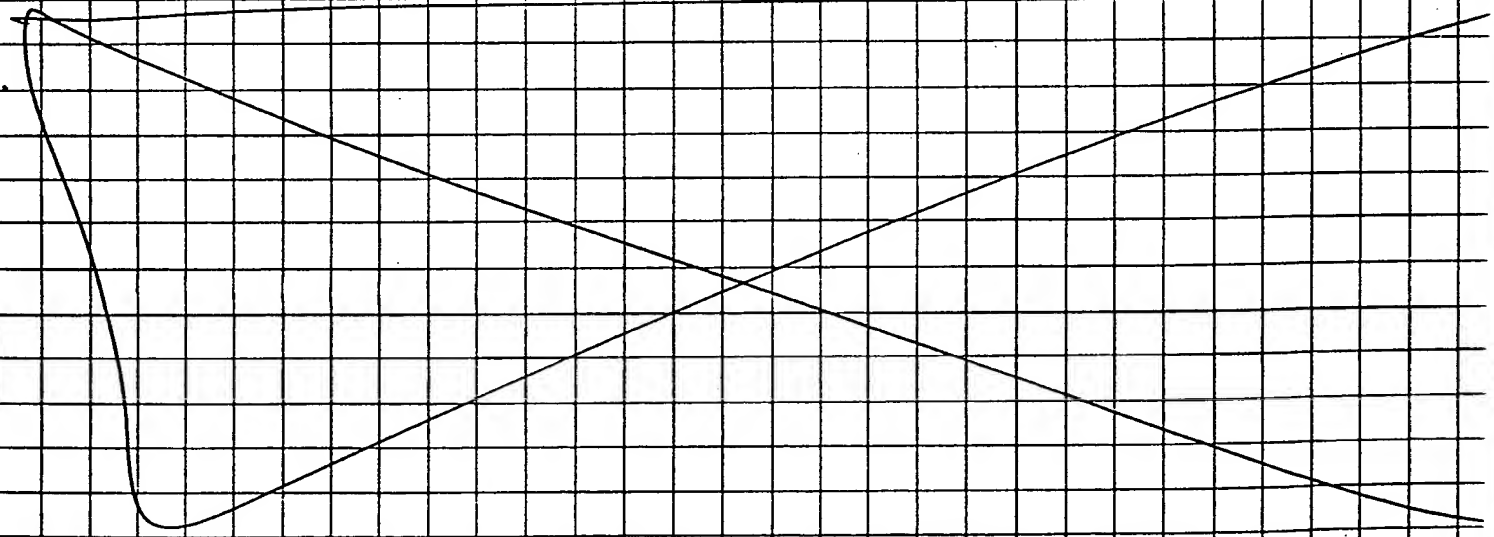
PCR's — primer sets

1 = P13 / P28
2 = P13 / P29
3 = P13 / P30
4 = P13 / P31
5 = P13 / P14 (cold control)

Per rxn mixed
10 µl 10x buffer
16 µl dNTP's
1 µl 1st primer
1 µl 2nd primer
2 µl 1:10 PRK5/HPTK6 (~90ng)
69 µl H_2O
1 µl Taq
100

+100 µl oil

Ran same conditions as on p. 17 of



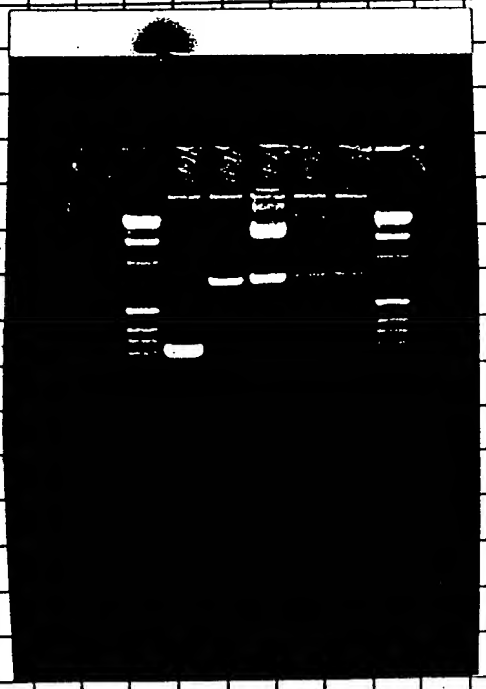
To Pa

Witnessed & Understood by me, | Date | Invented by WMA Barron | Recorded by | Dat[redacted]

rom Page No. 61

Extracted o/N PCR's 1x 100µl CHCl3 each

Ran 20µl each on 2% agarose (1x TBE)



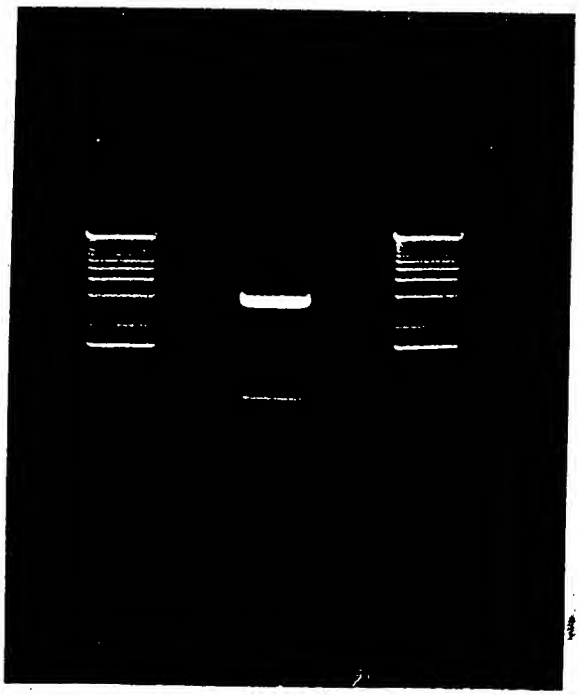
This is very frustrating!

On 4/16 I will re-do using various template

To Page No

Witnessed & Understood by me,	Date	Invented by WM Bauman	Date [redacted]
		Recorded by	

Want to prepare SacI/StuI Frag of pRK5/HPTK
Digested & ran on 0.7% agarose (1x TBE)
cut out indicated band
Freeze/squeeze
(-20°C → 2 hrs, spin
3 templates to be used
for Fusion PCR
will be HPTK6 Insert
HPTK6 IRb frag &
λ Hep 3B #6



Ran These 3 templates
w/ following primers

13,28
13,29
13,30
13,31
13,14 (cont)

different 5' primers { 16,28 / 16,29 / 16,30 / 16,31 / 16,14

= 30 rxns total

To Page

Witnessed & Understood by me,	Date	Invented by Will Bacon	Date [redacted]
		Recorded by [redacted]	

From Page No. 63

Per rxn mixed

10µl 10x buffer
16µl dNTPs
1µl 1st primer
1µl 2nd primer
1µl diluted template
70.5µl H_2O
0.5µl Taq
100
+100µl 0.1

Conditions

94°C	3'	1 cycle
55°C	30"	
72°C	30"	
98°C	30"	4 cycles
55°C	30"	
72°C	30"	
96°C	30"	20 cycles
55°C	30"	
72°C	30"	w/ 1sec auto-ext
72°C	10'	1 cycle
4°C	soak	

Ran + Then stored -20°C until [redacted].

To Page No.

Witnessed & Understood by me, | Date | Invented by [redacted] | Date [redacted]

Recorded by

om Page No. 65

Did Magic PCR preps on FUS 28, 29, 30 & 31 PCR's (4 total
RD'd [redacted] entire samples
w/ AvrII & BstEII
Stored -20°C O/N.

To Page No. 66

Witnessed & Understood by me,	Date	Invented by [redacted] Bacon	Date [redacted]
		Recorded by	

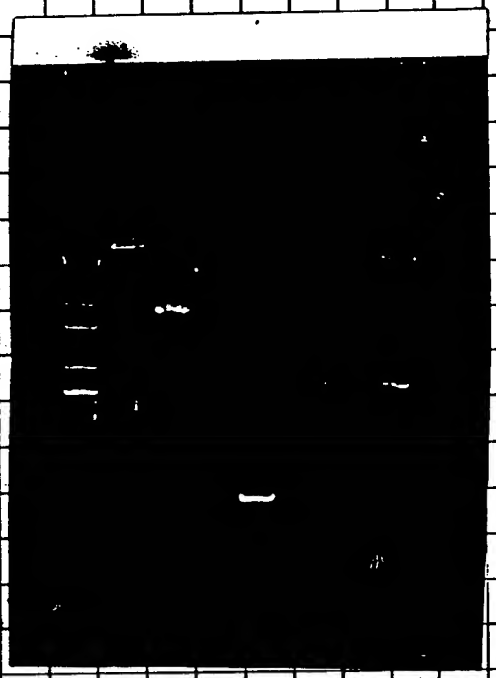
TITLE

From Page No. 16

Ran digested FNS PCR's on 1% LMP agarose (1 x TBE

along w/ AvrII/EcoRI digested Hep3B #6

BstEII/EcoRI digested SKO/CH2CH3



PCR frags did not dige

No 5' insert is visib

Re-ran fusion PCR's a

before => O/N.

To Pag

Witnessed & Understood by me,

Date

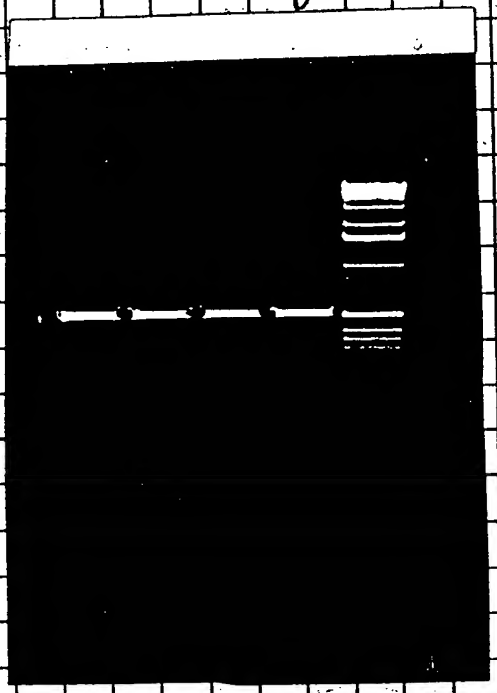
Invented by

Date

From Page No. 67

Extracted O/N PCR's 1x100µl $CHCl_3$

Ran 50% of each (50µl) on 0.8% LMP agarose



cut out indicated bands

RS'd each in 44µl TE

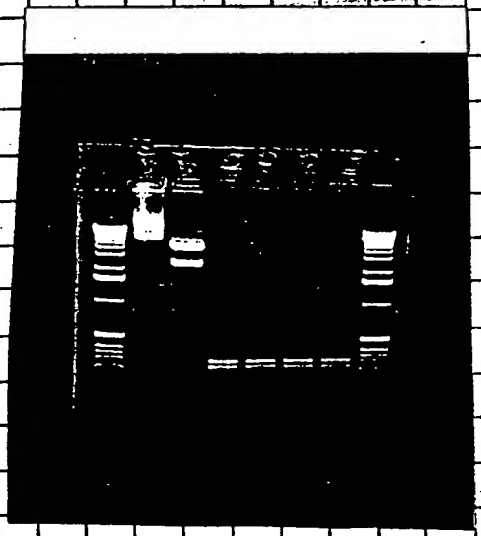
Arr II RD's

Per rxn Mixed

44µl PCR DNA
5µl 10x NEB #2
1µl Arr II
50

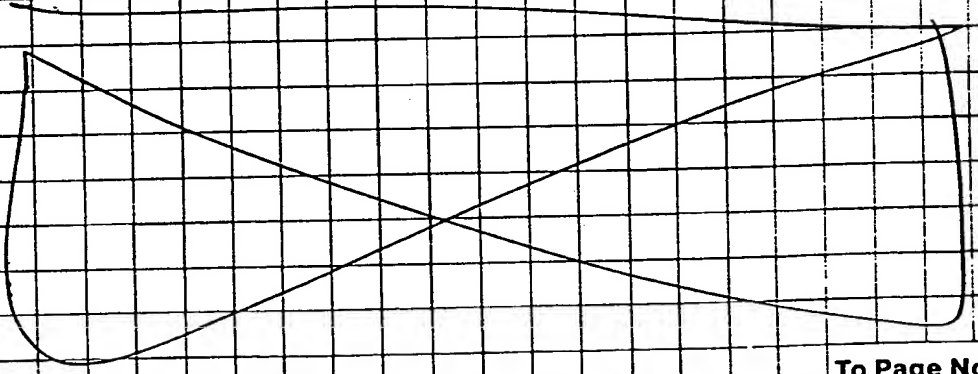
Inc 37°C ~ 1.5hrs

Removed 10µl each → Ran on 1% agarose (1x TBE)



Digests successful
Did Magic clean up's on remaining samples

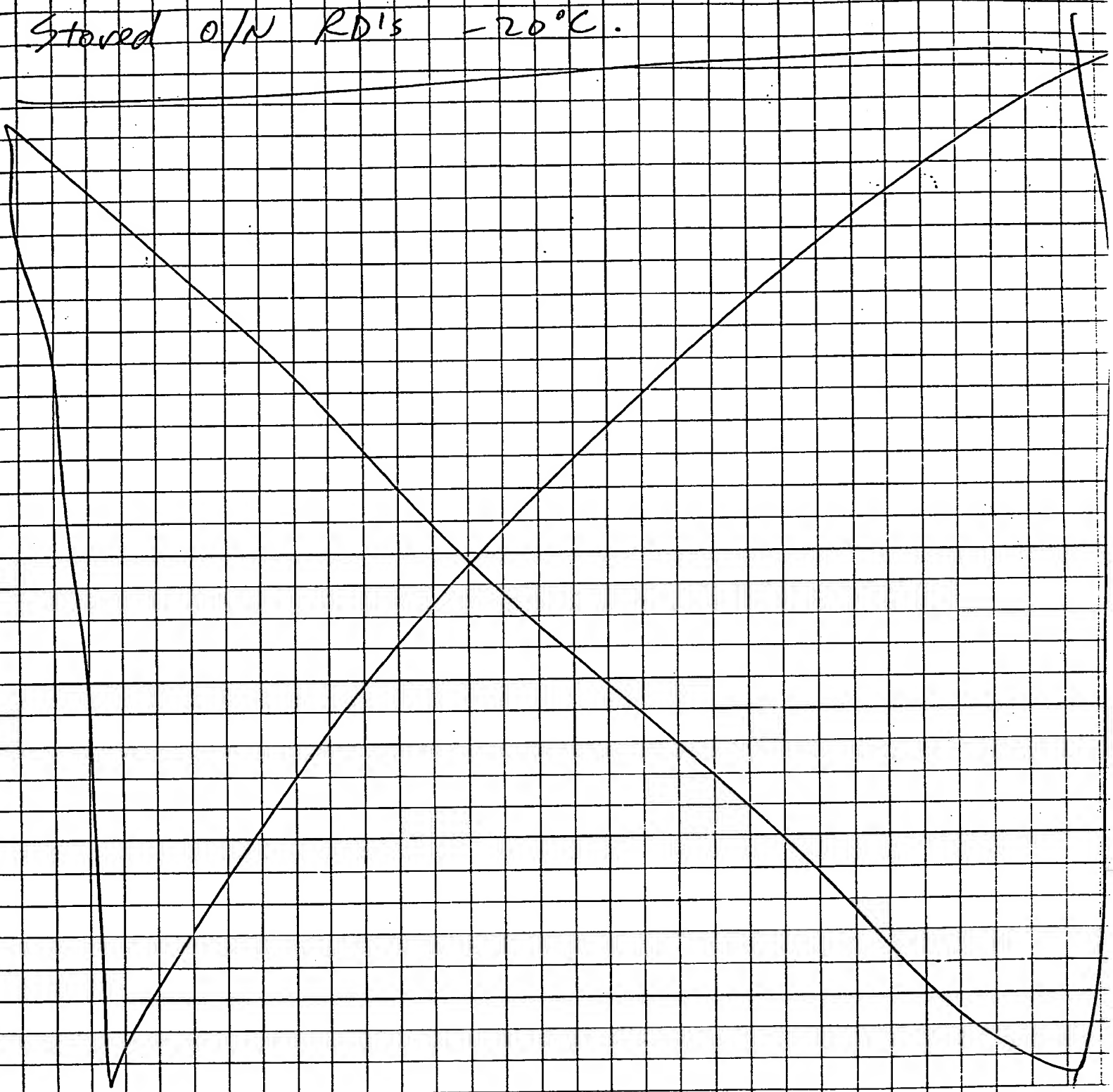
Ran BstE II RD O/N 60°C.



To Page No.

Witnessed & Understood by me, | Date | Invented by LILIA BACON | Date [redacted]

Stored O/N RD's -20°C.

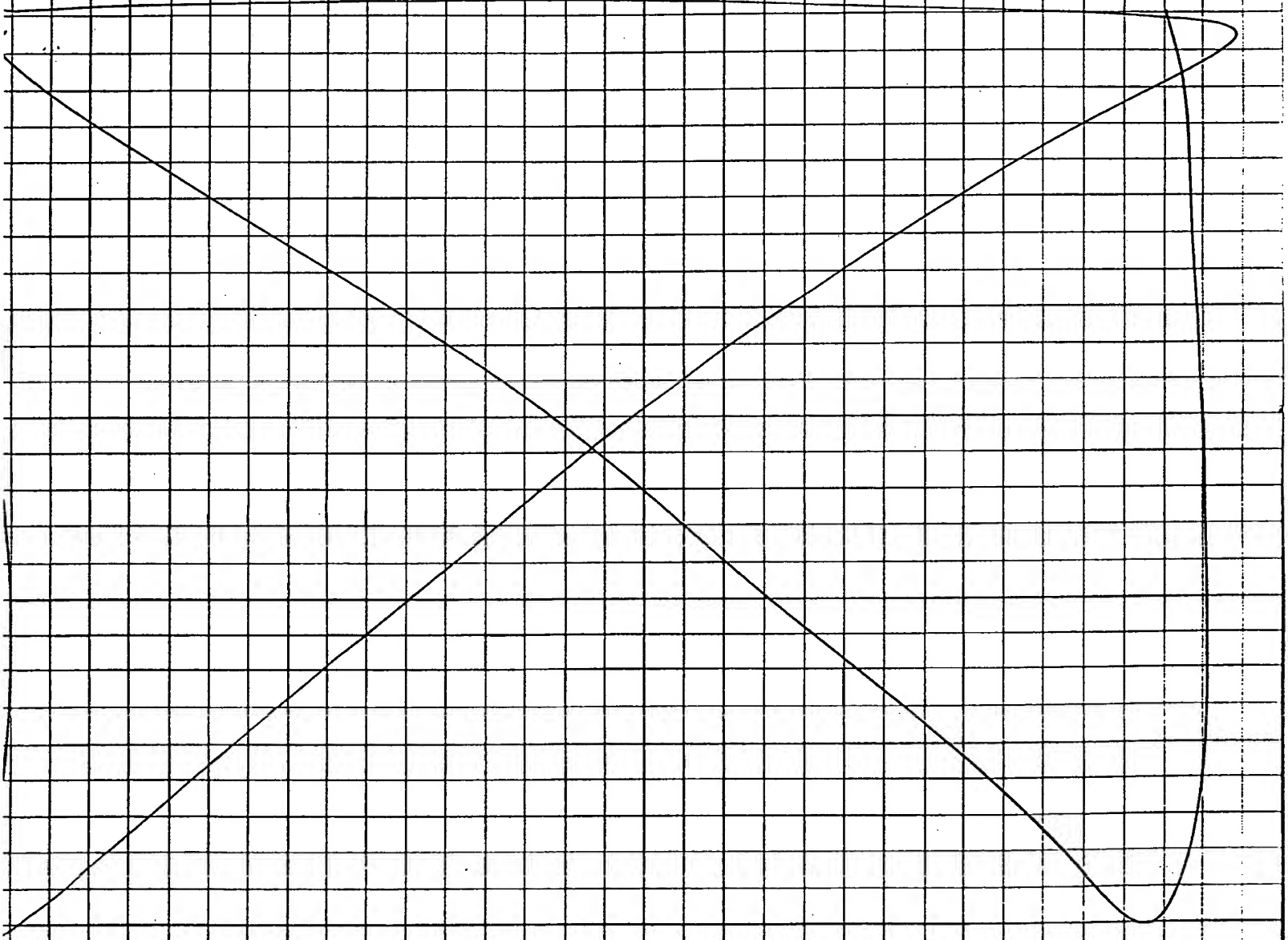


To Page No

Witnessed & Understood by me,	Date	Invented by	Date [redacted]
		Recorded by Will Benson	[redacted]

Page No. 69

an frozen FMS PCR RD's on 1% LMP agarose (1 x TBE)
- Gel not photographed
- Isolated ~300 bp bands
- Loaded in eppendorf tubes -20°C O/N.



To Page No. 71

ssed & Understood by me, | Date | Invented by [redacted] | Date [redacted]

From Page No. 20

Did magic PCR preps on frozen FUS 3' pieces
Recovered each in 50µl TE

Did 3 part Ligs for FUS 29 & FUS 30 w/ 5' ins (Hi & Lo conc insert)
& BK-/CH2CH3 vect (plus vect alone)
= 5 Ligations total.
Inc all (5) 12.5°C O/N.

To Page

Witnessed & Understood by me, | Date | Invented by | Date [redacted]
Recorded by

From Page No. 72

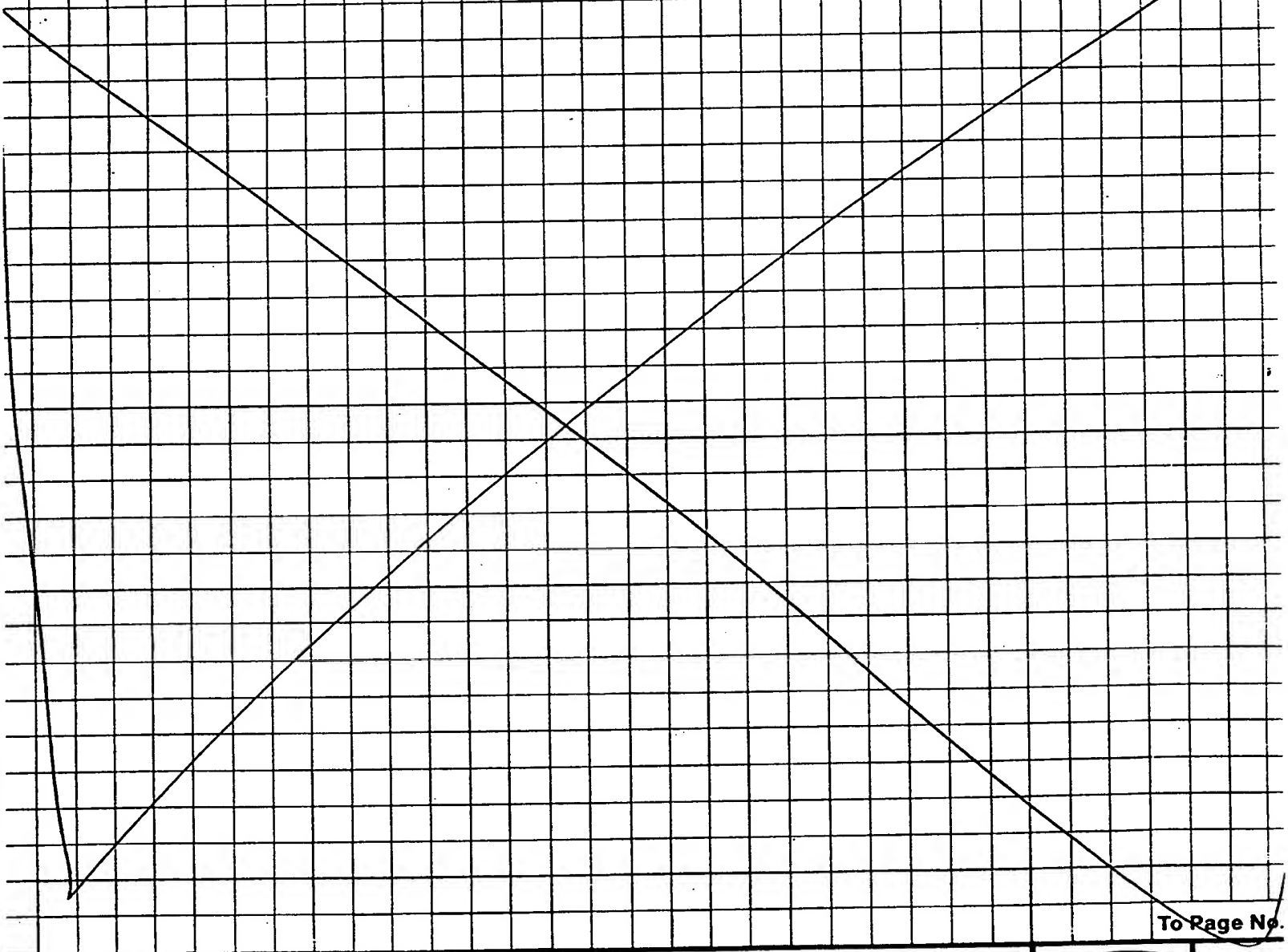
#	29/Hi	29/Lo	30/Hi	30/Lo	Co
	50	14	15	18	46

Checked transformation plates

Started 60 x 5ml LB cont 50 MP's + masters

(30 on each Fus 29 & Fus 30).

Inc all 37°C O/N.



To Page No.

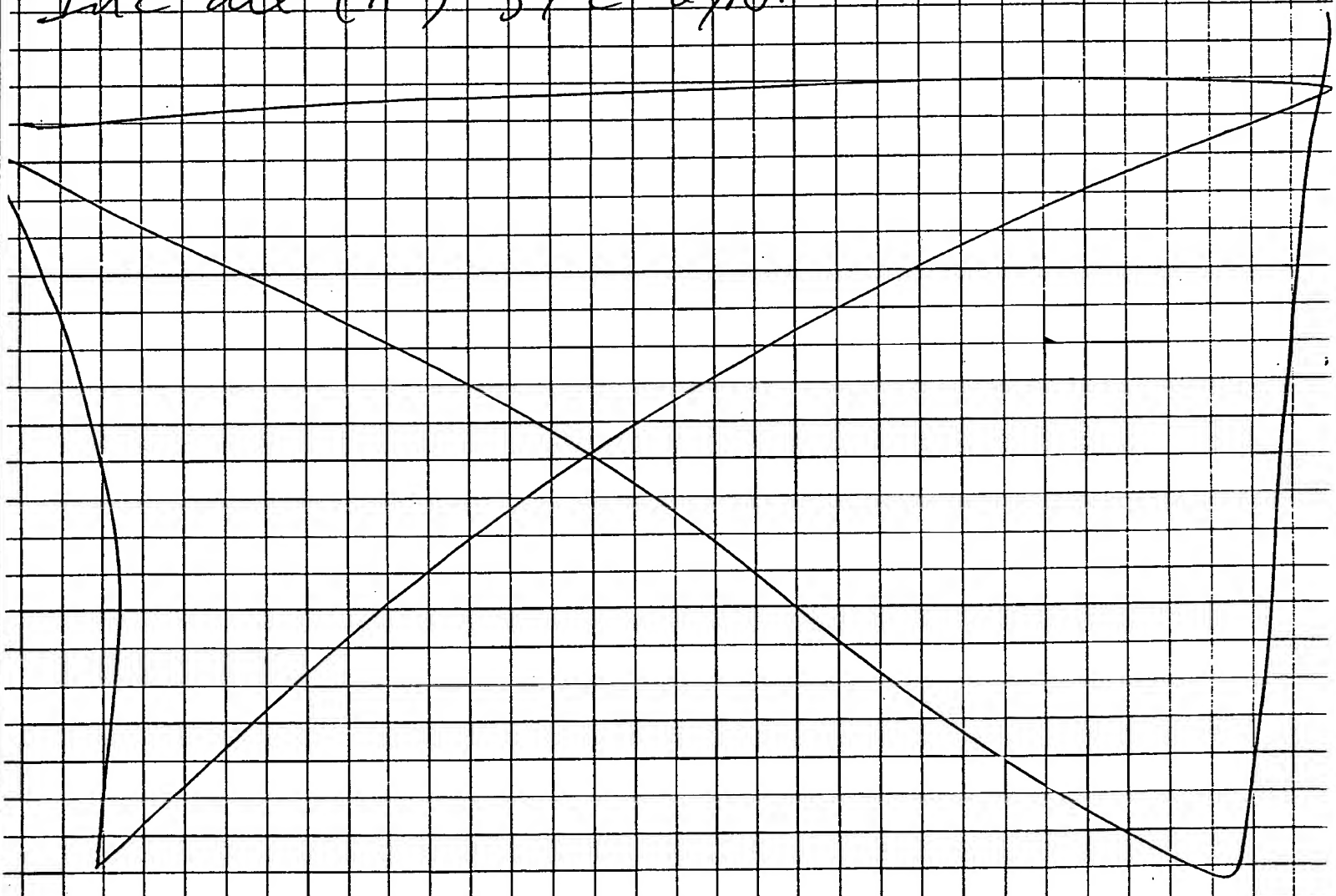
Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by W.M. Barron	[redacted]

m Page No. 71

Transformed O/N FUS Ligations into competent E. coli.

Plated each onto 5 x 100mm LB carb 50 plates (plated control onto 1 plate).

Inc all (11) 37°C O/N.



To Page No. 7

Witnessed & Understood by me,

Date

Invented by

Recorded by W M Bacon

Date [redacted]

From Page No. 73

Ran MP cultures (SK-1CH2CH3/HPTK6 Fusion) in Autogen (stored master 4°C)
Collected each in ~200µl TE
RD's (60 total)
Per rxn:

5µl MP DNA
2µl 10x B
0.5µl HindIII
1µl RNase A
11.5µl H_2O
20

Inc 37°C O/N

SAT- moved RD's to -20°C

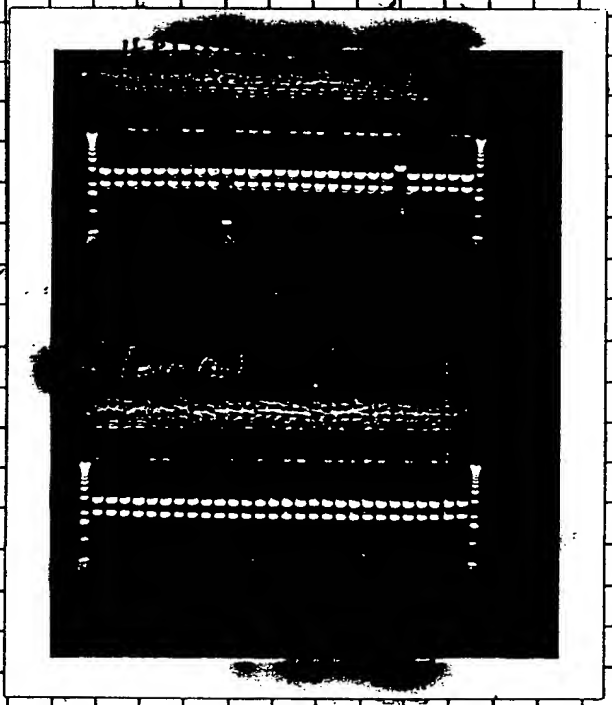
To Page No. 75

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		WM Bacon	[redacted]

TITLE _____ Project No. 1713 Book No. 17522

From Page No. 74

Ran FMS RD's on 0.7% agarose (1x TBE)



only 2 of 56 are negative

~~Blank~~

Did magic clean-ups on

29 #'s 1, 2, 3, 4, 5, 7, 8 + 9

30 #'s 31, 32, 33, 34, 35, 36, 37,

Collected each in 50µl TE

Denatured 10µl each in 100µl 0.1 N NaOH

Neutralized & EtOH ppt'd O/N -

To Page No. 7

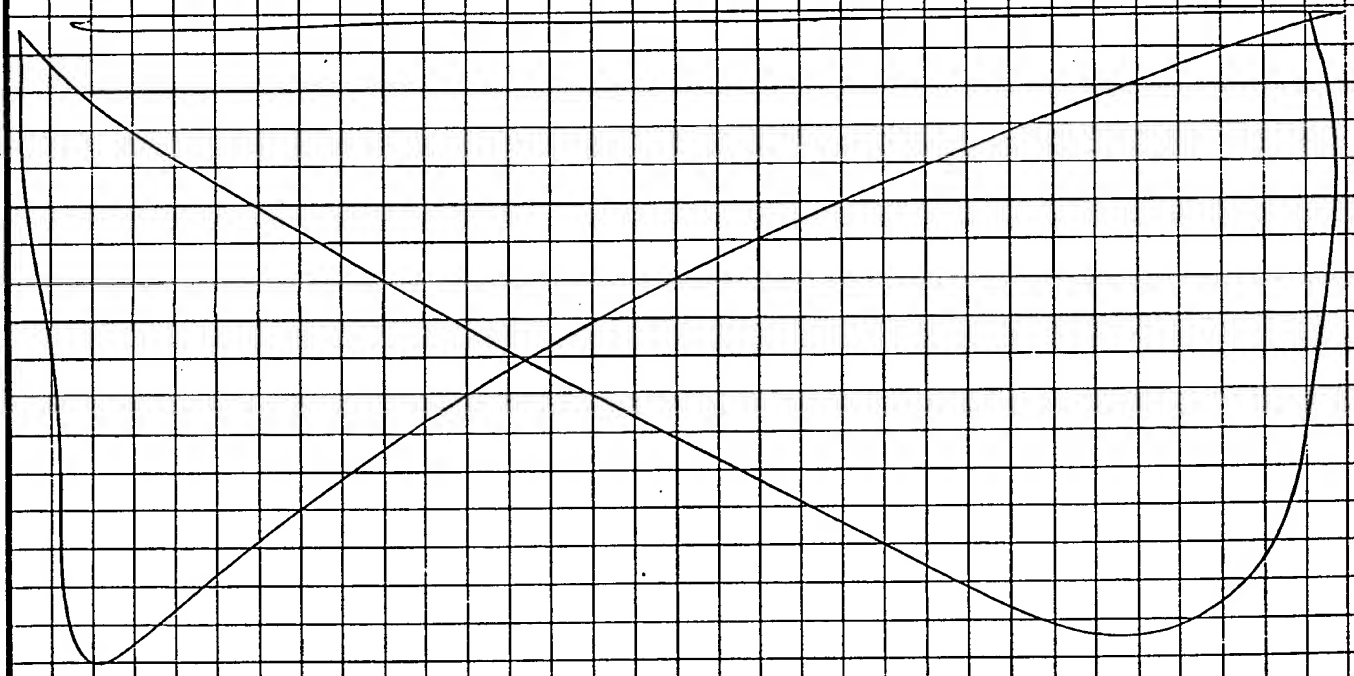
Witnessed & Understood by me,	Date	Invented by [redacted]	Date [redacted]
		Recorded by [redacted]	

Project No. 713
78 Book No. 17522 TITLE

From Page No. 77

Did sequencing runs on FHS 29 (8 triplates)
using CH2 CH3 sequencing primers
(used sequenase kit w/ 35-dATP)
Dried down samples → stored -20°C O/N.

Poured 2 sequencing gels
1 wedge + 1 flat
Stored RT O/N.



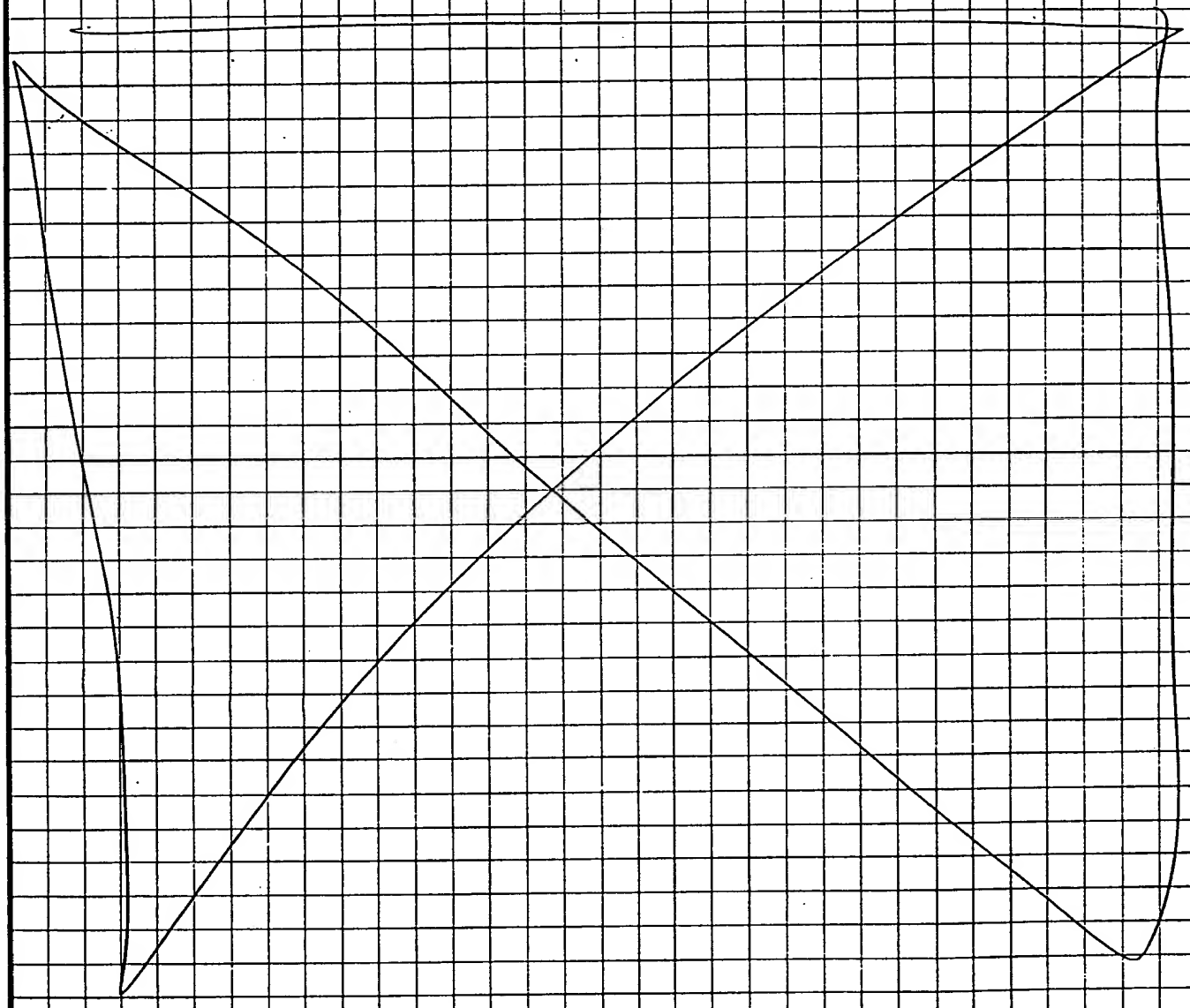
To Page No. 79

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by WM Bacon	[redacted]

TITLE

From Page No. 78

Ran sequencing gels on FWS 29/5'-CH2CH3
Dried & X/R'd both RT O/N.



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No. 79

Witnessed & Understood by me,	Date	Invented by Will Bacon	Date [redacted]
		Recorded by	

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Book No. 17522 TITLE

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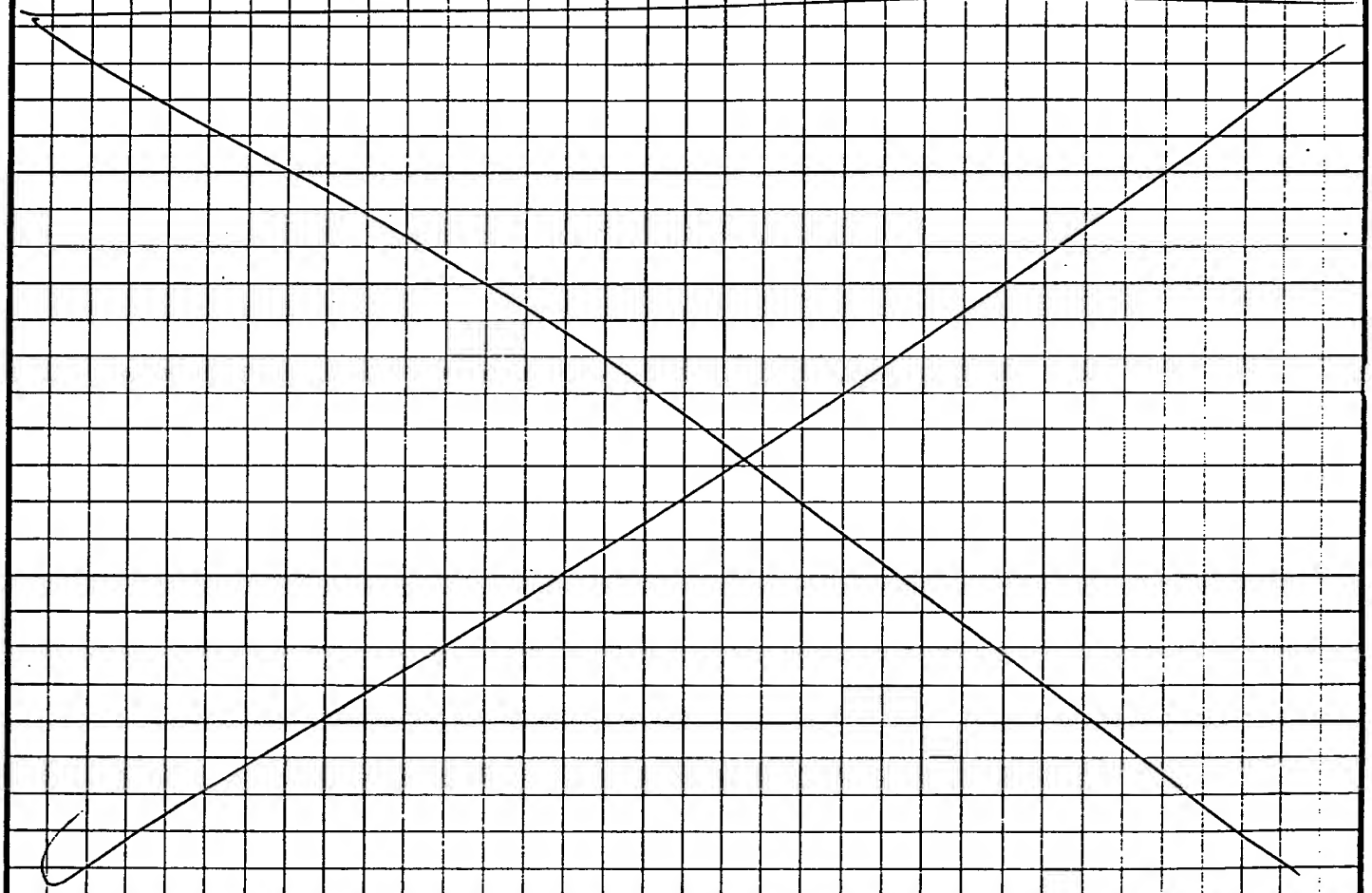
Developed o/N sequencing H/R.
I have correct sequence for SK- CHRCH3/FUS 29 #1
Ready to subclone to pSV15.ID.LL for
mammalian expression.



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Witnessed & Understood by me,	Date	Invented by WM Barron	Date [redacted]
		Recorded by	